Applicant: James Harris ylward

Serial No.: to be assigned Filed: June 21, 2001

Page : 3

(c) is capable of inhibiting the growth of at least one cell line selected from the group consisting of MM96L, MM229, MM220, MM537, MM2058, HeLa, B16, LIM1215, A549, MCF7, MCC16 and Colo16.

ney's Docket No.: 07404-003001

34. The method of claim 33, wherein the Euphorbia species is selected from the group consisting of Euphorbia peplus, Euphorbia drummondii and Euphorbia hirta.

35. The method of claim 33, wherein the compound comprises a composition selected from the group consisting of a jatrophane, a jatrophane derivative and a pharmaceutically acceptable salt of a jatrophane or a jatrophane derivative.

- 36. The method of claim 35, wherein the compound comprises a composition comprising a jatrophane ring conformation.
- 37. The method of claim 36, wherein the jatrophane ring containing composition is present in two diastereomeric conformations.
- 38. The method of claim 36, wherein the jatrophane ring containing composition is present in one diastereomeric conformation.
- 39. The method of claim 38, wherein the diastereomeric conformation is a conformation II.
- 40. The method of claim 36, wherein the composition comprising a jatrophane ring conformation comprises a nicotinate moiety.
- 41. The method of claim 36, wherein the composition comprising a jatrophane ring conformation comprises a benzoate moiety.

Applicant: James Harris Serial No.: to be assigned

Filed

: June 21, 2001

Page

42. The method of claim 36, wherein the composition comprising a jatrophane ring conformation comprises a iso-butyrate moiety.

ney's Docket No.: 07404-003001

43. The method of claim 33, wherein the jatrophane derivative comprises an ester derivative.

44. The method of claim 33, wherein the jatrophane derivative comprises an acetylated derivative.

45. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 1 position of a moiety selected from the group consisting of a -H and a -OAc.

46. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 2 position of a moiety selected from the group consisting of a -H, a -OAc and a CH₃.

47. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 3 position of a moiety selected from the group consisting of a -OH, a -OAc, a -OiBu (O(CH₃)₂CHCO), a -OCinn, a -OBz, a -OBzOCH₂CO, and a -PhCH₂CH₂CO₂.

- 48. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 4 position of an -H.
- 49. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 5 position of a moiety selected from the group consisting of a -OAc, a -OiBu (O(CH₃)₂CHCO), -OMeBu (OCH₃CH₂CH(CH₃)CO) and a -OAcAc.

Applicant: James Harris Serial No.: to be assigned

Filed

: June 21, 2001

Page

: 5

50. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 6 position of a moiety comprising an exocyclic double bond.

ney's Docket No.: 07404-003001

- 51. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 7 position of an -H₂, a -OAc, a -OiBu (O(CH₃)₂CHCO), a -OmeBu (OCH₃CH₂CH(CH₃)CO), a -OPr, a -OCOiPr and a -OCOEt.
- 52. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 8 position of an -H₂, a -OH, a -OAc, a -OiBu (O(CH₃)₂CHCO), a -OmeBu (OCH₃CH₂CH(CH₃)CO), a -OBz and a -OAng.
- 53. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 9 position of an -OH, a -OAc (-OCH₃CO), a -OCinn (OPhCHCHCO), a -ONic ($C_5H_4NCO_2$) and an = O.
- 54. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 10 position of a - $(CH_3)_2$.
- 55. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 11 and carbon 12 positions comprising a double bond between carbon 10 and carbon 11.
- 56. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 13 position of a -(CH₃).
- 57. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 14 position of an -H, an -OH, a -OAc (OCH $_3$ CO) and an = O.

Applicant: James Harris

Serial No.: to be assigned Filed : June 21, 2001

Page

58. The method of claim 36, wherein the jatrophane derivative comprises a substitution in the jatrophane ring carbon 15 position of an -OH and a -OAc (OCH₃CO).

ney's Docket No.: 07404-003001

- 59. The method of claim 35, wherein the composition comprises a 2,3,5,7,15pentaacetoxy-9-nicotinoyloxy-14-oxojatropha-6(17),11E-diene (jatrophane 1) or a pharmaceutically acceptable salt.
- 60. The method of claim 35, wherein the composition comprises a 2,5,7,8,9,14hexaacetoxy-3-benzoyloxy-15-hydroxy-jatropha-6(17),11E-diene (jatrophane 2) or a pharmaceutically acceptable salt.
- 61. The method of claim 35, wherein the compound comprises a 2.5.14triacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutyroyloxy-9-nicotinoyloxyjatropha-6(17), 11Ediene (jatrophane 3) or a pharmaceutically acceptable salt of these.
- 62. The method of claim 35, wherein the compound comprises a 2,5,9,14tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutyroyloxyjatropha-6(17),11E-diene) (jatrophane 4) or a pharmaceutically acceptable salt of these.
- 63. The method of claim 35, wherein the compound comprises a 2,5,7,14tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-9-nicotinoyloxyjatropha-6(17),11E-diene (jatrophane 5) or a pharmaceutically acceptable salt of these.
- 64. The method of claim 35, wherein the compound comprises a 2,5,7,9,14pentaacetoxy-3-benzoyloxy-8,15-dihydroxyjatropha-6(17),11E-diene (jatrophane 6) or a pharmaceutically acceptable salt of these.
- 65. The method of claim 33, wherein the compound comprises a composition selected from the group consisting of a pepluane, a pepluane derivative and a pharmaceutically acceptable salt of a pepluane or a pepluane derivative.

Filed

: June 21, 2001

Page

66. The method of claim 65, wherein the pepluane derivative comprises an ester derivative.

ney's Docket No.: 07404-003001

67. The method of claim 65, wherein the pepluane derivative comprises an acetylated derivative.

68. The method of claim 65, wherein the pepluane derivative comprises a substitution in a position in a pepluane skeleton selected from the group consisting of

an -H₂ or an -OAc (-OCH₃CO) at a carbon 1 position;

a -CH₃ and an -H at a carbon 2 position;

an -OBz at a carbon 3 position;

an -H at a carbon 4 position:

an -OAc (-OCH₃CO) at a carbon 5 position;

a -CH₃ or an -CH₂OAc at a carbon 6 position;

an -H₂ at a carbon 7 position;

an -OAc (-OCH₃CO) or a double bond to C12 at a carbon 8 position;

an -OAc (-OCH₃CO) or a double bond to C18 at a carbon 9 position;

a -CH₃ and an -OAc (-OCH₃CO), a -CH₃, or a double bond to C11 at a carbon 10

position;

an -H₂ or a double bond to C10 at a carbon 11 position;

an -H or a double bond to C8 at a carbon 12 position;

a -CH₃ at a carbon 13 position;

an -OAc (-OCH₃CO) at a carbon 14 position;

an -OH at a carbon 15 position; and,

an -H or an - H_2 at a carbon 18 position.

69. The method of claim 65, wherein the pepluane comprises a composition selected from the group consisting of a 5,8,9,10,14-pentaacetoxy-3-benzoyloxy-15hydroxypepluane, a derivative of a 5,8,9,10,14-pentaacetoxy-3-benzoyloxy-15-hydroxypepluane

oseesor.ose

and a pharmaceutically acceptable salt of a 5,8,9,10,14-pentaacetoxy-3-benzoyloxy-15hydroxypepluane.

70. The method of claim 33, wherein the compound comprises a compositionselected from the group consisting of a paraliane, a paraliane derivative and a pharmaceutically acceptable salt of a paraliane or a paraliane derivative.

ney's Docket No.: 07404-003001

71. The method of claim 70, wherein the paraliane derivative comprises an ester derivative.

72. The method of claim 70, wherein the paraliane derivative comprises an acetylated derivative.

73. The method of claim 70, wherein the paraliane derivative comprises a substitution in a position in a paraliane skeleton selected from the group consisting of

an -H, an -H₂ or an -OAc (-OCH₃CO) at a carbon 1 position;

a -CH₃ and an -H or a -CH₃ and an -OAc (-OCH₃CO) at a carbon 2 position;

an -OBz at a carbon 3 position;

an -H at a carbon 4 position;

an -OAc (-OCH₃CO) at a carbon 5 position;

a -CH₃ or a -CH₂OAc at a carbon 6 position;

an -H₂ at a carbon 7 position;

an -H or an -OAc (-OCH₃CO) at a carbon 8 position;

an = O at a carbon 9 position;

a - $(CH_3)_2$ at a carbon 10 position;

an -H₂ at a carbon 11 position;

an -H at a carbon 12 position;

a -CH₃ at a carbon 13 position;

an -OAc (-OCH₃CO) at a carbon 14 position; and,

an -OH at a carbon 15 position.

TŲ

Applicant: James Harris ylward

Serial No.: to be assigned Filed: June 21, 2001

Page : 9

74. The method of claim 33, wherein the compound comprises a composition selected from the group consisting of a angeloyl-substituted ingenane, a angeloyl-substituted ingenane derivative and a pharmaceutically acceptable salt of a angeloyl-substituted ingenane or a angeloyl-substituted ingenane derivative.

ney's Docket No.: 07404-003001

- 75. The method of claim 74, wherein the angeloyl-substituted ingenane derivative comprises an ester derivative.
- 76. The method of claim 74, wherein the angeloyl-substituted ingenane derivative comprises an acetylated derivative.
- 77. The method of claim 74, wherein angeloyl-substituted ingenane is selected from the group consisting of a 20-O-acetyl-ingenol-3-angelate, an acetylated derivative of a 20-O-acetyl-ingenol-3-angelate and an ester derivative of a 20-O-acetyl-ingenol-3-angelate.
- 78. A method of stimulating the immune system, the method comprising administering to the subject an effective amount of at least two compounds,

wherein the two compounds are derived from an extract from the sap of a species of *Euphorbia*, wherein the compounds

- (a) are extractable from the Euphorbia sap in the presence of about 95% v/w ethanol,
- (b) have cell inhibiting or retarding activity which is neither destroyed by acetone nor by heating at about 95°C for about 15 minutes, and
- (c) are capable of inhibiting the growth of at least one cell line selected from the group consisting of MM96L, MM229, MM220, MM537, MM2058, HeLa, B16, LIM1215, A549, MCF7, MCC16 and Colo16.
- 79. The method of claim 78, wherein the compounds are selected from the group consisting of a jatrophane, a jatrophane derivative, a pharmaceutically acceptable salt of a

Applicant: James Harris vlward

Serial No.: to be assigned Filed : June 21, 2001

Page

: 10

jatrophane, a pepluane, a pepluane derivative, a pharmaceutically acceptable salt of a pepluane, a paraliane, a paraliane derivative, a pharmaceutically acceptable salt of a paraliane, an angeloylsubstituted ingenane, an angeloyl-substituted ingenane derivative and a pharmaceutically acceptable salt of an angeloyl-substituted ingenane.

ney's Docket No.: 07404-003001

80. The method of claim 78, wherein the compounds are selected from the group consisting of a 5,8,9,10,14-pentaacetoxy-3-benzoyloxy-15-hydroxypepluane (pepluane), a derivative of a 5,8,9,10,14-pentaacetoxy-3-benzoyloxy-15-hydroxypepluane, a 2,3,5,7,15pentaacetoxy-9-nicotinoyloxy-14-oxojatropha-6(17),11E-diene (jatrophane 1), a derivative of a 2,3,5,7,15-pentaacetoxy-9-nicotinoyloxy-14-oxojatropha-6(17),11E-diene, a 2,5,7,8,9,14hexaacetoxy-3-benzoyloxy-15-hydroxy-jatropha-6(17),11E-diene (jatrophane 2), a derivative of a 2,5,7,8,9,14-hexaacetoxy-3-benzoyloxy-15-hydroxy-jatropha-6(17),11E-diene, a 2,5,14triacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutyroyloxy-9-nicotinoyloxy-jatropha-6(17),11Ediene (jatrophane 3), a derivative of a 2,5,14-triacetoxy-3-benzoyloxy-8,15-dihydroxy-7isobutyroyloxy-9-nicotinoyloxy-jatropha-6(17),11E-diene, a 2,5,9,14-tetraacetoxy-3benzoyloxy-8,15-dihydroxy-7-isobutyroyloxyjatropha-6(17),11E-diene (jatrophane 4), a derivative of a 2,5,9,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutyroyloxyjatropha-6(17),11E-diene, a 2,5,7,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-9-nicotinoyloxyjatropha-6(17),11E-diene (jatrophane 5), a derivative of a 2,5,7,14-tetraacetoxy-3-benzoyloxy-8,15dihydroxy-9-nicotinoyloxyjatropha-6(17),11E-diene, a 2,5,7,9,14-pentaacetoxy-3-benzoyloxy-8,15-dihydroxyjatropha-6(17),11E-diene (jatrophane 6), a derivative of a 2,5,7,9,14pentaacetoxy-3-benzoyloxy-8,15-dihydroxyjatropha-6(17),11E-diene, a 20-O-acetyl-ingenol-3angelate, a derivative of a 20-O-acetyl-ingenol-3-angelate and pharmaceutically acceptable salt of one or any combination of these compounds.

- 81. The method of claim 78, wherein the compounds are provided in the form of a chemical fraction obtained from the sap of a species of Euphorbia.
- 82. The method of claim 33, wherein the compound further comprises a betaalanine betaine or a hydroxy-dimethyl proline.

Applicant: James Harris ylward

Serial No.: to be assigned Filed : June 21, 2001

Page

: 11

83. The method of claim 33, wherein the compound is capable of inhibiting or retarding the growth of MM96L cells.

ney's Docket No.: 07404-003001

- 84. The method of claim 33, wherein the compound is capable of inducing differentiation of MM96L cells.
- 85. The method of claim 33, wherein the compound is capable of inducing normal melanocytes to proliferate.
- 86. The method of claim 33, wherein the compound is capable of inducing T cells to proliferate.
- 87. The method of claim 33, wherein the compound is capable of inducing the expression of G-CSF.
- 88. The method of claim 33, wherein the compound is capable of inducing the expression of major histocompatibility complex (MHC) molecules.
- 89. The method of claim 33, wherein the compound is capable of recruiting a natural killer cell to a region of application of the compound.
- 90. The method of claim 33, wherein the compound is capable of a T cell to a region of application of the compound.
- 91. The method of claim 33, wherein the compound is provided in the form of a composition comprising a pharmaceutically- or cosmetically-acceptable carrier.

Applicant: James Harris ylward

Serial No.: to be assigned Filed: June 21, 2001

Page : 12

92. The method of <u>claim 91</u>, wherein the pharmaceutically- or cosmetically-acceptable carrier is selected from a β -alanine betaine hydrochloride and a t-4-hydroxy-N,N-dimethylproline.

ney's Docket No.: 07404-003001

93. A method of recruiting an immune cell to a region of application of a compound, the method comprising administering an effective amount of the compound to the region,

wherein the compound is derived from an extract from the sap of a species of *Euphorbia*, wherein the compound

- (a) is extractable from the Euphorbia sap in the presence of about 95% v/w ethanol,
- (b) has cell inhibiting or retarding activity which is neither destroyed by acetone nor by heating at about 95°C for about 15 minutes, and
- (c) is capable of inhibiting the growth of at least one cell line selected from the group consisting of MM96L, MM229, MM220, MM537, MM2058, HeLa, B16, LIM1215, A549, MCF7, MCC16 and Colo16.
- 94. The method of claim 93, wherein a natural killer cell is recruited to the region of application of the compound.
- 95. The method of <u>claim 93</u>, <u>wherein a T cell is recruited to the region of application of the compound.--</u>